

by strikethroughs and matter that has been added to the specification is indicated by double underlining.

Please amend the specification as follows:

On page 1, please replace the paragraph beginning "This application is" with the following paragraph:

This application is a continuation of U.S. Patent Application Serial No. 09/273,685, filed March 22, 1999, now U.S. Patent No. 6,015,561, which in turn is a division of U.S. Patent Application Serial No. 08/488,161 filed June 7, 1995, which issued as U.S. Patent No. 5,885,577 on March 23, 1999, which in turn is a continuation-in-part of co-pending U.S. Patent Application Serial No. 08/310,192 filed September 21, 1994, now abandoned, the entire contents of which are incorporated herein by reference.

On page 9, please replace the paragraph beginning "The present invention" with the following paragraph:

The present invention relates to abtides. As used herein, the term "abtides" refers to peptides that mimic the binding specificity of a larger molecule such as an antibody or receptor. Abtides specifically bind to a ligand of interest, in which the ligand is a specific binding partner of the larger molecule (e.g. antibody or receptor). To identify the abtides of the present invention, peptide libraries are screened in a two-step process. The first screening step uses an antibody (or antigen-binding derivative thereof) or receptor (or ligand-binding derivative thereof) as a first target ligand. This step identifies peptide sequences termed "epitopes" or "mimetopes" which specifically bind the first target ligand. In the case where an antibody or derivative thereof is used as the first target ligand, a mimotope will often resemble, either functionally in terms of its binding capability and/or structurally in terms of its amino acid sequence, the epitope recognized by the antibody used as the first ligand. An epitope or mimotope is then used as a second target ligand in a second screening step to identify a peptide sequence that specifically binds the epitope or mimotope. Such peptides are known as "abtides." Surprisingly, it was found by the current inventor and is demonstrated herein that abtides possess binding specificities strikingly similar to those possessed by the first target ligands (usually antibodies or receptors) described above.

A2
On page 10, please replace the paragraph beginning "4. FIGURE LEGENDS" with the following:

A3
4. BRIEF DESCRIPTION OF THE DRAWINGS

On page 11, please replace the paragraph beginning "Figure 5 shows" with the following paragraph:

A4
Figure 5 shows the binding of biotinylated mimetopes to immobilized abtides. □ represents binding of mimotope peptide Biotin-LYANPGMYSRLHSPA-NH₂ (SEQ ID NO: 20) to 7E11-C5 abtide clone 14; ○ represents binding of mimotope peptide Biotin-LYANPGMYSRLHSPA-NH₂ (SEQ ID NO: 20) to 7E11-C5 abtide clone 17; ◇ represents binding of mimotope peptide Biotin-GMYSRLH-NH₂ (a portion of SEQ ID NO: 20) to 7E11-C5 abtide clone 14; △ represents binding of mimotope peptide Biotin-GMYSRLH-NH₂ (a portion of SEQ ID NO: 20) to 7E11-C5 abtide clone 17. See Section 6.1.2.2 for details.

On page 12, please replace the paragraph beginning "Figure 10 schematically" with the following paragraph:

A5
Figure 10 schematically illustrates the construction of the R26 TSAR library. The R26 expression library was constructed essentially as described for the TSAR-9 library that is described in PCT publication WO 94/18318, dated August 18, 1994, except for the modifications depicted in Figure 10. The oligonucleotide assembly process depicted in Figure 10 results in expression of peptides with the following amino acid sequence: S(S/R)X₁₂πAδX₁₂SR (SEQ ID NO: 25), where π = S, P, T or A; and δ = V, A, D, E OR G ctgtgcctcgagB(NNB)₁₂Nccgcgg is SEQ ID NO: 87; ctgtgctctaga(VNN)₁₂VNccgcgg is SEQ ID NO: 88, tcgagB(NNB)₁₂Nccgcgg is SEQ ID NO: 89; ctatg(VNN)₁₂VNccgcgg is SEQ ID NO: 90; SHSS(S/R)X₁₂πAδX₁₂SRPSRT is SEQ ID NO: 91.

On page 12, please replace the paragraph beginning "Figure 11 schematically" with the following paragraph:

A6
Figure 11 schematically illustrates the construction of the D38 TSAR library. The D38 expression library was constructed essentially as described for the TSAR-9 library that is described in PCT publication WO 94/18318, dated August 18, 1994, except for the

Alex Clark

modifications depicted in Figure 11. GTGTGTCTGCGAGN(NNB)₂₀NACGCCAN is SEQ ID NO: 92; GTTGTGTCTAGA(VNN)₁₅VNTGGCGTN is SEQ ID NO: 93; TCGAGN(NNB)₂₀NACGCCAN is SEQ ID NO: 94; CTAGA(VNN)₁₅VNTGGCGTN is SEQ ID NO: 95; HSS(S/R)X₂₀(Y/H/N/D)A(I/M/T/N/K/S/R)X₁₅SR is SEQ ID NO: 96.

On page 12, please replace the paragraph beginning "Figure 12 schematically" with the following paragraph:

Figure 12 schematically illustrates the construction of the DC43 TSAR library.

P7

The DC43 expression library was constructed essentially as described for the TSAR-9 library that is described in PCT publication WO 94/18318, dated August 18, 1994, except for the modifications depicted in Figure 12. GTGTGTCTCGAGN(NNB)₂₀GGTTGTGGT is SEQ ID NO: 97; GTTGTGTCTAGA(VNN)₂₀ACCACAAACC is SEQ ID NO: 98; TCGAGN(NNB)₂₀GGTTGTGGT is SEQ ID NO: 99, CTAGA(VNN)₂₀ACCACAAACC is SEQ ID NO: 100; HSS(S/R)X₂₀GCGX₂₀GCGX₂₀SR is SEQ ID NO: 101.

On page 12, please replace the paragraph beginning "Figure 13 schematically" with the following paragraph:

P8

Figure 13 schematically illustrates the oligonucleotides used to construct the polymorphic epithelial mucin (PEM) abtide saturation mutagenesis TSAR library (See Section 6.2.2). GAPVWRGNPRWRGPFFKWPAGCNGPMCNTGTPARGGSRNNGP is SEQ ID NO: 51; ggsgccscgtstgsagsggsaasccscggtsgsagsgccggsggttsaastgscgsGGCTGCAGGG is SEQ ID NO: 102, sggscctttscgsgasccscscgscggsgttaasgtsttscasatggsccttCCCGCAGCC is SEQ ID NO: 103.

On page 20, please replace the paragraph beginning "Therefore, it is" with the following paragraph:

P9

Therefore, it is contemplated that the most preferred binding domains for identifying the abtides of the present invention will be those from biologically expressed random peptide libraries in which the displayed peptide is 20 or greater amino acids in length. Examples of such random peptide libraries are the TSAR libraries, described in PCT publication WO 91/12328, dated August 22, 1991, and PCT publication WO 94/18318, dated

AA
ant

August 18, 1994

✓

On page 27, please replace the paragraph beginning "As used in" with the following paragraphs:

A10 In an embodiment, a molecule comprises a peptide or a binding portion thereof which binds to a ligand of interest, which peptide is identified by a method comprising: screening a random peptide library with a ligand of interest, said ligand of interest being a peptide having a length of between 5 and 40 amino acids, to identify a peptide that specifically binds to the ligand of interest, in which the ligand of interest is also specifically bound by an antibody or a receptor.

In another embodiment, a molecule comprises a peptide which binds to a substance of interest, which peptide is identified by a method comprising: screening a random peptide library with a ligand, said ligand being a peptide of 36 amino acids or fewer, in which the ligand is an epitope of an antigen that is specifically bound by an antibody or in which the ligand represents the portion of a receptor-ligand that is responsible for the specific binding of the receptor to the receptor-ligand. As used in the present invention, a ligand is a substance for which it is desired to isolate a specific binding partner from a peptide library. A ligand can function as a lock, *i.e.*, a large polypeptide or protein analogous to a lock into which a smaller specific binding partner fits as a key; or a ligand can function as a key which fits into and specifically binds a larger binding partner or lock.

On page 28, please replace the paragraph beginning "A preferred method" with the following paragraph:

A11 A preferred method for identifying abtides comprises screening a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising (a) a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600, and optionally, (b) an effector domain encoded by an oligonucleotide sequence which is a protein or peptide that enhances expression or detection of the binding domain. Screening is done by contacting the plurality of heterofunctional fusion proteins with a ligand under conditions conducive to ligand

P11

binding and then isolating the fusion proteins which bind to the ligand. The methods of the invention further preferably comprise determining the nucleotide sequence encoding the binding domain of the heterofunctional fusion protein identified to determine the DNA sequence that encodes the binding domain and simultaneously to deduce the amino acid sequence of the mimotope used in the second screen. Nucleotide sequence analysis can be carried out by any method known in the art, including but not limited to the method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of SEQUENASE™ T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699; U.S. Biochemical Corp.), or TAQ™ polymerase, or use of an automated DNA sequenator (*e.g.*, Applied Biosystems, Foster City, CA).

A12

On page 41, please amend the paragraph beginning "(a) Polystyrene microtiter wells", as follows:

- (a) Polystyrene microtiter wells (Flow Laboratory) are coated overnight at room temperature with 100 µl of a solution of a molecule comprising an abtide at a concentration of 1 mg/ml in phosphate buffered saline (PBS).
- (b) Coating solution is discarded and wells are blocked for 1-2 hours at room temperature with 300 µl of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) with 0.05% of "TWEEN™" 20 (polyoxyethylenesorbitan monolaurate) (PBS-TWEEN™ buffer).
- (c) 150 µl of sample (suspected of containing an analyte the presence or amount of which it is desired to measure) diluted in 1% BSA-PBS is added per well. Wells are incubated 1 hour at room temperature.
- (d) Wells are washed 4 times with PBS-TWEEN™ buffer.
- (e) 100 µl of horseradish peroxidase conjugated monoclonal antibody specific for the analyte in 1% BSA-PBS is added per well. The concentration of the monoclonal antibody can be from about 10 ng/ml to 10 mg/ml. Wells are incubated 1 hour at room temperature.
- (f) Wells are washed 6 times with PBS -TWEEN™ buffer.
- (g) 100 µl of ABTS® Boehringer Mannheim (2,2'-Azino-di-[3-ethylbenzthiazidine sulfonate (6)] crystallized

AB11

diammonium salt working solution is added per well. ABTS® stock solution is prepared at 15 mg/ml in dH₂O. To make the working solution, 200 µl of this ABTS® stock is diluted into 10 ml of citrate phosphate buffer (17 mm citric acid, 65 mm dibasic sodium phosphate) and 10 µl 30% H₂O₂.

- (h) The absorbance of each well is measured at 405 nm in a microtiter plate reader (Dynatech MR600, Dynatech Corp., Alexandria, VA).

On page 45, please replace the paragraph beginning "Abtides may be" with the following paragraph:

AB3
Abtides may be linked to chelators such as those described in U.S. Patent No. 4,741,900 or U.S. Patent No. 5,326,856. The abtide-chelator complex may then be radiolabeled to provide an imaging agent for diagnosis or treatment of disease. The abtide may also be used in the methods that are disclosed in co-pending U.S. patent application serial no. 08/127,351, now U.S. Patent No. 5,449,761, for creating a radiolabeled peptide for use in imaging or radiotherapy. This application contains a review of methods of using peptides in imaging agents.

On page 49, please replace the paragraph beginning " By way of" with the following paragraph:

AB4
By way of example but not limitation, peptides can be synthesized on an Applied Biosystems Inc. ("ABI") model 431A automated peptide synthesizer using the "Fastmoc" synthesis protocol supplied by ABI, which uses 2-(1H-Benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate ("HBTU") (R. Knorr et al., 1989, Tet. Lett., 30:1927) as coupling agent. Syntheses can be carried out on 0.25 mmol of commercially available 4-(2',4'-dimethoxyphenyl-(9-fluorenyl-methoxycarbonyl)-aminomethyl)-phenoxy polystyrene resin ("Rink resin" from Advanced ChemTech) (H. Rink, 1987, Tet. Lett. 28:3787). Fmoc amino acids (1 mmol) are coupled according to the FASTMOCTM protocol. The following side chain protected Fmoc amino acid derivatives are used: FmocArg(Pmc)OH; FmocAsn(Mbh)OH; FmocAsp('Bu)OH; FmocCys(Acm)OH; FmocGlu('Bu)OH; FmocGln(Mbh)OH; FmocHis(Tr)OH; FmocLys(Boc)OH; FmocSer('Bu)OH;

P14 cont

FmocThr('Bu)OH; FmocTyr('Bu)OH. [Abbreviations: Acm, acetamidomethyl; Boc, tert-butoxycarbonyl; 'Bu, tert-butyl; Fmoc, 9-fluorenylmethoxycarbonyl; Mbh, 4,4'-dimethoxybenzhydryl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Tr, trityl].

On page 53, please replace the paragraph beginning "In order to" with the following paragraph:

P15

In order to identify abides mimicking binding specificity of monoclonal antibody 7E11-C5, monoclonal antibody 7E11-C5 was used as the target ligand in a first screening of the TSAR-9 library (see Kay et al., 1993, Gene 128:59-65 and PCT publication WO 94/18318, dated August 18, 1994). The following screening procedure was used. First, 7E11-C5 was bound to a well of a microtiter plate. 7E11-C5 at a concentration of 11.2 mg/mL in phosphate buffered saline (PBS), pH 6.0, was diluted to 100 µg per mL in 0.1x PBS pH 7.2. One hundred microliters (100 µL) of this dilution was added to one well of a microtiter plate, and allowed to incubate for 1-6 hours at room temperature or overnight at 4° C. After incubation, the well was washed at least 4 times with a blocking buffer which consisted of either 1% bovine serum albumin (BSA) in PBS, 1% non-fat dry milk (NFDM) in PBS, or 0.1% TWEEN™ in either 1% BSA in PBS or 1% NFDM in PBS. Two hundred microliters of the blocking buffer was then added to the well and allowed to incubate for at least an hour at room temperature.

On page 54, please replace the paragraph beginning "Next, an aliquot" with the following paragraph:

P14

Next, an aliquot of the TSAR-9 library was added to the well containing bound 7E11-C5. An aliquot of the library containing 10^{10} phage particles was added to the well and allowed to incubate for at least 1 hour at room temperature. This resulted in the binding to the plate of those phage containing binding domains that bind to 7E11-C5. After an hour, the well was washed extensively with either 1% bovine serum albumin (BSA) in PBS, 1% non-fat dry milk (NFDM) in PBS, or 0.1% TWEEN™ in either 1% BSA in PBS or 1% NFDM in PBS.

On page 56, please replace the paragraph beginning "Using the above" with the following paragraph:

P17

Using the above procedures, nine different phage were isolated that expressed peptides containing binding domains that were capable of binding monoclonal antibody 7E11-C5. Molecules comprising these binding domains are thus mimetopes of the antigen recognized by the monoclonal antibody 7E11-C5. The binding domains of the peptides expressed by the nine phage were sequenced according to standard methods of DNA sequencing (SEQUENASE™, U.S. Biochemical Corp., Cleveland, OH). The determination of those DNA sequences allowed the determination of the amino acid sequences of these mimetopes. These sequences are shown in Table 1. Examination of these amino acid sequences showed that they shared a common motif of MYxxLH (SEQ ID NO. 10).

On page 60, please replace the paragraph beginning "In some cases," with the following paragraph:

P18

In some cases, these abtides were used in a dot blot experiment. In those cases, 1 µL of a 1 mg/mL solution of the 38-residue abtides was spotted onto nitrocellulose (0.2 µm or 0.45 µm, Schleicher & Schuell, Keene, NH) strips or circles. After drying (about ½ hour), the nitrocellulose was blocked for 1 hour in a solution of 1% BSA in PBS. The nitrocellulose was then allowed to incubate in approximately 5 mL of a solution of 0.1 mg/mL of a biotinylated 7E11-9.5 mimotope peptide (biotin-LYANPGMYSRLHSPA)-NH₂ (SEQ ID NO: 20). This mimotope peptide was one of those described in Section 6.1.1 above that were synthesized based upon the nine peptides that were identified in the screening of Section 6.1.1 above. After an hour, the nitrocellulose was washed approximately 5 times with a solution of 1% BSA in PBS. A 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was then added and allowed to incubate for 1 hour, after which the nitrocellulose was again washed extensively. Finally, a solution of 5-bromo-4-chloro-3-indolyl phosphate (0.15 mg/mL) and nitro blue tetrazolium (0.3 mg/mL) (Sigma, St Louis, MO) (BCIP/NBT) was added as an enzyme substrate. Color was allowed to develop and the absorbance at 405 nm was read.

On page 65, please replace the paragraph beginning "For biodistribution studies," with the following paragraph:

P19

For biodistribution studies, abtides were modified at their amino termini with the chelator diethylene-triamine-pentaacetic acid anhydride (DTPA-A) (Sigma, St. Louis,

*P1
Cont*

MO). Approximately 2 mg of each abtide was initially dissolved in an appropriate volume of 0.1% acetic acid and then 1 mL of 0.1 M sodium bicarbonate, pH 8.0, was added. Two mg of DTPA-A was suspended in 100 µL of dimethylsulfoxide (DMSO), and 10 µL of the abtide solution added to this DTPA-A suspension. After 5 min incubation at room temperature, the suspension was filtered through a 0.2 µm ACRODISC™ polyvinylidene difluoride (PVDF) sample filter(ACRODISC™, Gelman Sciences, Inc., Ann Arbor, MI), and purified using a Superose-12 FPLC column (Pharmacia, Piscataway, NJ) with PBS as the running buffer.

Modified peptides were stored frozen at -20° C or -70° C.

On page 76, please replace the paragraph beginning ‘Negative Binding Sequences with the following paragraph:

Negative Binding Sequences

A20
MPI **GAPVWRGNPRWRGPFFKWPAGCGNGPMCNTFTPARGGSRNNGP** (SEQ ID NO: 51)

- E3 **GTRVPPGFALRGGRDGLSWAGCGKA**P**IISKTYTSARGRSRKKG**S**** (SEQ ID NO: 77)
- E15 **RSAVSEGKPREIVPGGCMWPGCGNGRKSNTLHGPEQFQEIEP** (SEQ ID NO: 78)
- E24 **SSGVNGKPRS**W**APDALNGGC**N**IQFANTITPD**R**GGSCNQTL** (SEQ ID NO: 79)
- E27 **GSSVCGGQPSGRGF**G**GLPGPGCGNGPTSNTLTSARGGF**N**KGL** (SEQ ID NO: 80)
- E37 **GAPL**W**QGDPADEV**L**GGSMIP**G**CGIGALSQTFT**T**PGGS**R**KNVT** (SEQ ID NO: 81)
- E43 **AGREL**R**QDE**E**GGAGAD**V**ARLREG**P**ICST**F**TPARGG**S**CPS**G**L** (SEQ ID NO: 82)
- E49 **QARVSMAISCRSGPSDLMH**Q**GCG**Y**GP**R**CNPD**T**TD**S**GG**H**T**N**TP** (SEQ ID NO: 83)
- E60 **GDPECRG**K**PRGRWT**G**SLACT**G**CG**N**GP**N**SKICTRARG**V**SRN**K**GP** (SEQ ID NO: 84)

- E72 STPGCSGYSGSGDPRCLTCTACGNGHTRKTLTPAHRSTHKEP (SEQ ID NO: 85)
- E34 GQPECRITSGCCGTDGNKWLGCVKVDMCNTLNPAVGCHGTNGS (SEQ ID NO: 86)
- E83 REPVVGGKPWCRGPGGLWRGCGKSQFDKIITLSRDNRRDKRP (SEQ ID NO: 23)

On page 77, please replace the paragraph beginning "When the sequences" with the following paragraph:

p21
When the sequences shown in Table 7 are compared (see particularly the amino acid residues marked in boldface type), it is possible to determine the influence of particular amino acid residues at specific positions in the sequence on a peptide's ability to bind to PEM. Abtides that bind to PEM can be characterized by the formula:

R₁R₂R₃R₄R₅R₆R₇R₈R₉R₁₀R₁₁R₁₂R₁₃R₁₄R₁₅R₁₆R₁₇R₁₈R₁₉R₂₀R₂₁R₂₂R₂₃R₂₄R₂₅R₂₆R₂₇R₂₈R₂₉
R₃₀R₃₁R₃₂R₃₃R₃₄R₃₅R₃₆R₃₇R₃₈R₃₉R₄₀R₄₁R₄₂R₄₃ (SEQ ID NO: 24)

where:

R₁= G, C, E, or V, preferably G;

R₂= A, S, P, or L, preferably A;

R₃= P, T, H, or L, preferably P;

R₄= L, M, Q, G, A, or S;

R₅= W or Y, preferably W;

R₆= S, C, K or T, preferably S;

R₇= E, S, C, D, V, or R;

R₈= N, H, K, S, or E;

R₉= L, H, R, N, Q, T, or G;

R₁₀= W, P, R, T, or D, preferably W;

R₁₁= W, C, V, L, or G, preferably W;

R₁₂= S, T, M, or H, preferably S or T;

R₁₃= G;

R₁₄= S, A, G, N, Q, or H, preferably S;

R₁₅= W, H, G, A, or R;

R₁₆= G, T, E, P, V, or W, preferably G;

R₁₇= V, F, W, K, or A;

R₁₈= K, Q, D, E, R, or L, preferably K;

R₁₉= R, F, or S, preferably R;

R₂₀= P, S, I or H, preferably P;

R₂₁= G;

R₂₂= C;

R₂₃= G;

R₂₄= D, S, T, N, or H;

R₂₅= G, D, L, or R;

R₂₆= P or S, preferably P;

R₂₇= M, S, D, I, L, or R;

R₂₈= G, W, C, L, F, Y, or T, preferably G or W;

R₂₉= S, N, V, F, H, or R;

R₃₀= N, A, S, M, or R, preferably N;

R₃₁= F, Q, P, or V, preferably F;

R₃₂= S, V, I, K, A, or S;

R₃₃= P, A, N, or Y, preferably P;

R₃₄= G, N, or L;

R₃₅= K, R, C, Q or L, preferably K or R;

R₃₆= V, K, R, or A;

R₃₇= G, D, A, or E, preferably G;

R₃₈= S, T, P, Y or W; preferably S;

R₃₉= R, I, L, P, A or S;

R₄₀= N, K, or M, preferably N or K;

R₄₁= S, R, T, E, Q, P, Y or H;

R₄₂= G, A, S, D, N, P, Y, or K, preferably G;

R₄₃= P, H or A.